Brief Communication Communication brève

Treatment of diplomonad intestinal parasites with magnesium sulphate at a commercial rainbow trout *(Oncorhynchus mykiss)* facility

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Abstract — Rainbow trout (average weight of 2 g) in fresh water experienced high mortality and were infected with a diplomonad intestinal parasite. Tanks of fish experienced an immediate reduction in mortality after an in-feed treatment with 3% Epsom salts for 2 d. Treatments had to be applied several times, but in each case there was a similar reduction in mortality.

Résumé — Traitement des parasites intestinaux diplomonades à l'aide de sulfate de magnésium dans une installation commerciale de truites arc-en-ciel *(Oncorhynchus mykiss).* Des truites arc-en-ciel (poids moyen de 2 g) élevées en eau douce ont connu une mortalité élevée et ont été infectées par un parasite intestinal diplomonade. Une réduction immédiate de la mortalité a été observée dans les bassins de poissons après un traitement dans l'alimentation de 3 % de sel d'Epsom pendant 2 jours. Les traitements ont dû être appliqués plusieurs fois, mais, dans chaque instance, il s'est produit une réduction semblable de la mortalité.

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Diplomonad flagellates have been reported in farmed salmonids in Europe and North America (1–4). These parasites typically belong to the genus *Spironucleus* (5,6). Speciation of these parasites is possible using molecular sequencing of the small subunit ribosomal RNA gene or by evaluating morphological characteristics using electron microscopy (6,7). However, these techniques are seldom used for diagnostic purposes.

Spironucleus spp. infect, predominantly, the anterior and posterior intestine, pyloric ceca, and gall bladder, but can also cause systemic infections (1,2,5,8,9). In rainbow trout, clinical signs include excessive nervousness that manifests as erratic swimming, fecal casts, and loss of appetite (4,5). Pathology associated with infections range from minimal to hemorrhages in the intestine, enteritis, and hepatocellular necrosis (4).

It is presumed that aquaculture fish become infected with these parasites from contaminated surface water sources. This parasite genus has a direct life cycle with an environmentally stable encysted stage and a mobile parasitic trophozoite stage (6). Parasites are transmitted from fish to fish via the fecal oral route or through contamination of the water (6).

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There are reports of efficacious chemotherapeutants against diplomonad flagellates *in vitro* (10) and *in vivo* (6,11–13). These pharmaceutical treatments (i.e., metronidazole, dimetridazole, and albendazole) are used for infections in ornamental fish (13) but are not approved for use in food fish (14,15). One exception is magnesium sulphate (Epsom salts), which has been used successfully to treat intestinal diplomand parasites in salmonids (16), but there is limited information in the literature on its application.

Case description

In May 2014, mortality started increasing (Figure 1) in a population of rainbow trout *(Oncorhynchus mykiss)* (~480 000 fish; average weight 2.0 g housed in 17 2-m^3 circular tanks) < 1 mo after an infection with *Flavobacteria psychrophilum.* The fish exhibited abnormal swimming behavior (flashing) similar to what would be expected with an infection by external parasites or due to poor water quality (e.g., high ammonia or gas super saturation). The fish were on a fresh water flow-through system supplied by a 24 m deep well (temperature 7°C). The hatchery was located in eastern Canada. Water quality testing by the producer at the start of the mortality event indicated that the system had normal levels for oxygen (100% saturation), ammonia (non-detectable), and pH (6.9). The producer applied a formalin bath treatment (167 ppm for 1 h) followed, the next day, by a chloramine-T treatment (10 ppm for 1 h) to address possible parasite and/or *F. psychrophilum* infections, but these bath treatments did not reduce mortality.

Twelve live fish were submitted for diagnostic testing at the Aquatic Diagnostic Services at the Atlantic Veterinary College, Charlottetown, Prince Edward Island. The fish arrived exhibiting abnormal swimming behavior and some fish had long

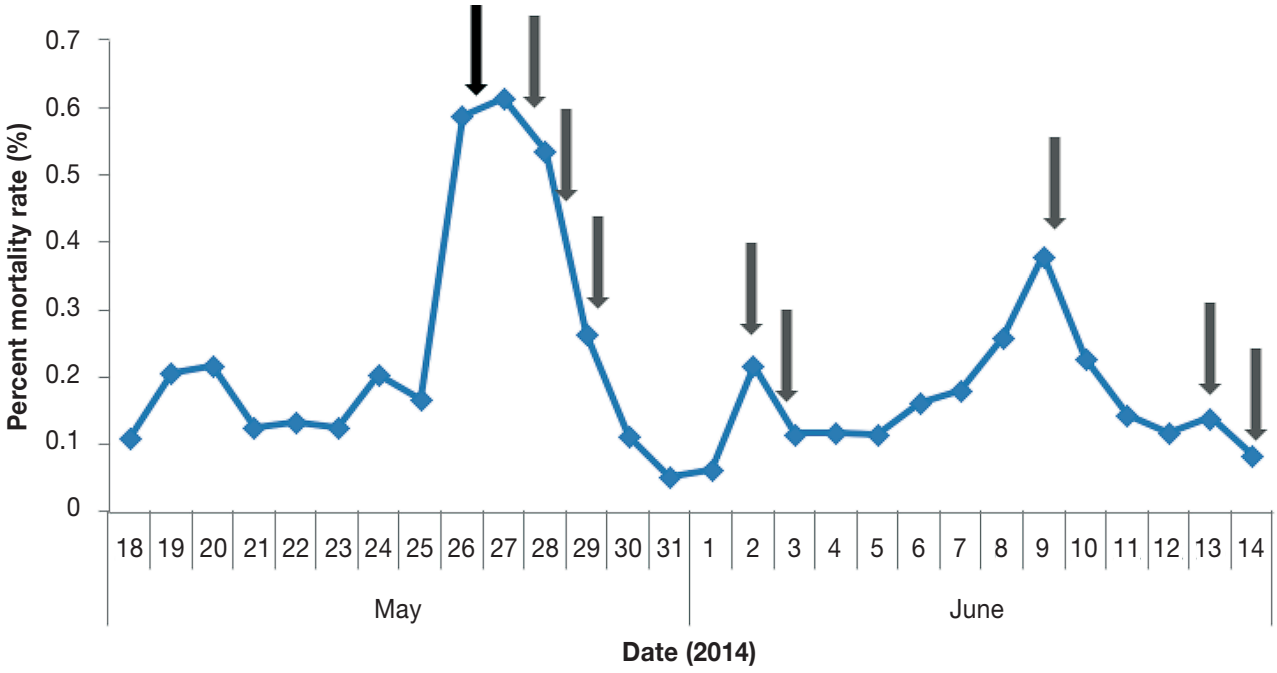

Figure 1. Daily rainbow trout mortality rate during and after the diagnosis and treatment of a diplomonad parasite. The black arrow indicates when the diagnosis was made and the grey arrows indicate when magnesium sulphate was included in the feed.

trailing white fecal casts. Fecal casts were also present in the transport container. Otherwise the fish appeared normal grossly.

Kidney and skin swabs were collected from 3 fish and plated on blood agar and Cytophaga medium. Cytophaga plates were incubated at 15°C and blood agar plates were incubated at 22°C. Wet mounts of the gills, skin, and intestinal content were prepared from 6 fish. In addition, we did wet mounts and imprints of 3 fecal casts collected from the transport container. Imprint smears were stained with Dip Quick Stain (Jorvet; Jorgensen Laboratories, Loveland, Colorado, USA).

Gill structure was normal and there were no signs of external parasites on either gill wet mount or skin scraping. All kidney tissue cultures were negative for aerobic bacteria after 5 d. One skin culture was positive and the bacterium was identified by MALDI-TOF Mass Spectrometry analysis as *F. psychrophilum*. Wet mounts and imprints of the lower intestinal content revealed diplomonad parasites (Figure 2), which were also observed on the imprint slides of the fecal casts.

Treatment with feed containing 3% Epsom salts (magnesium sulphate; Atoma Canada, Montreal, Quebec) (30 g Epsom salts per 1000 g of feed) was administered to 4 of the 17 tanks of fish for 2 consecutive days. The fish were fed at a rate of 2% body weight (BW) per day. Initially, only the 4 worst tanks were treated to monitor treatment effect; however, due to the immediate positive response of the fish in these tanks, all tanks with fish displaying the abnormal swimming behavior and white fecal casts were treated the next day. A salt (bath) treatment (~1%) was administered on the third day to reduce any parasites in the water column. This bath treatment was maintained for approximately 1 h.

Mortality started increasing again approximately 5 d after the initial treatment. The fish were treated again with Epsom salts in the feed for 2 d and again we observed an immediate decrease in mortality (Figure 1). Two additional treatments were necessary over the following 2-week period (Figure 1).

Discussion

Mortality in rainbow trout associated with intestinal diplomonad parasites responded positively to treatment with 3% in-feed Epsom salts (magnesium sulphate) at a feed rate of 2% body weight for 2 d, followed by a 1% salt bath treatment. This treatment has been recommended for aquarium fish and salmonids, but there are very few published reports of successful clinical applications (16,17). The advantages of using magnesium sulphate include the ease and low cost of treatment, as well as the low regulatory status of this product for use in food animals (14,15).

Magnesium sulphate acts as a laxative in fish. It stimulates cholecystokinin to be released from the mucosal enterocytes in the intestinal tract, which increases peristalsis and causes evacuation of the luminal contents, including associated parasites (18). It is also possible that magnesium sulphate has a direct effect on the parasite, as Sangmaneedet and Smith (10) found it to inhibit growth of *Spironucleus vortens (in vitro)* at concentrations > 60 mg/mL.

In this field case, fish were also exposed to a salt water bath treatment after 2 d of oral exposure to Epsom salts in the feed in an attempt to reduce the number of viable parasites in the water column. But, given that these fish were housed in a flow-through system and the frequency of water turnover was relatively high, the bath treatment was probably not necessary. Also, the concentration of salt may not have affected the encysted life stage of this parasite, which is relatively resistant to harsh environmental conditions (6).

The fact that the mortality rate decreased immediately after the application of the magnesium sulphate feed treatment on

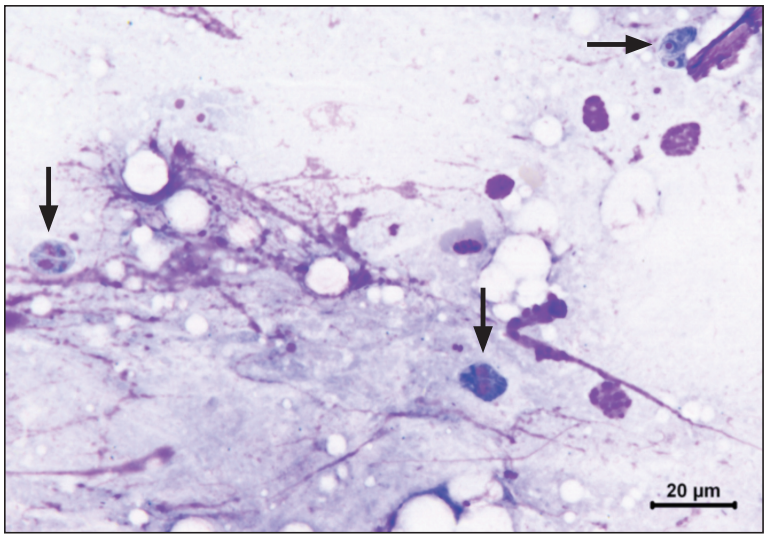

Figure 2. Dip Quick Stain of gut impression smear from rainbow trout. Arrows indicate the diplomonad protozoan parasite.

several occasions, but that we had to re-apply the treatment 4 times over the period of 1 mo, suggests the fish were getting re-infected with the parasite shortly after treatment and/or the parasite was not completely eliminated from the intestinal tract. Epsom salt therapy is transient in nature and will not provide the longer term effect typical of a systemic therapeutant.

Several chemical treatments such as metronidazole, the most commonly used, furazolidone, benznidazole, ronidazole, secnidazole, albendazole, aminosidine, diethylcarbamazine, and nitroscanate have been successfully used to treat intestinal diplomonad parasites in aquarium (non-food) fish (9–12,19) and may have required only 1 treatment. However, these therapeutants are not licensed for use in food animals.

The fact that the fish may have been stressed from a recent infection with *F. psychrophilum,* and the bacterium was isolated from the skin of 1 fish, may have played a role in fluctuating mortality observed during the study period and the need to re-treat; however, the immediate response to Epsom salt therapy suggests that the predominant issue with the population was the intestinal parasite infection and not *F. psychrophilum.*

The source of the intestinal parasite for this particular hatchery was difficult to identify, given that the water supply was from a 24-meter well. It is possible the parasite entered the groundwater in the spring, during periods of heavy rainwater run-off, which was reported by the farm manager shortly before the mortality event. The hatchery has reported this parasite in their fish around the same time of year (spring) in the past. If the source of the parasite is limited to a short period during the spring, it may be possible to control parasite-associated mortality with short, intermittent doses of Epsom salts in the feed during this vulnerable time. The in-feed Epsom salt treatment was palatable to the fish and resulted in a reduction of mortality immediately after the first day of administration. No side effects were observed from repeating the Epsom salt therapy.

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